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The Efficacy of Synbiotic Application in Broiler Chicken Diets, Alone or in Combination with Antibiotic Growth Promoters on Zootechnical Parameters

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ABSTRACT

In recent years, probiotics and synbiotics have gained considerable interest in poultry feeding as an alternative to antibiotics due to antibiotic resistance concerns. The objective of this dual study was to evaluate the efficacy of synbiotic supplementation alone or in combination with different Antibiotic Growth Promoters (AGPs), compared to the untreated control group of broiler chickens production performance. In the first experiment, a total of 1260 one-day-old male Ross 308 broiler chickens were randomly assigned to 7 diet treatments, with 6 replicates per diet treatment and 30 birds per replicate over a 42-day period. The diet treatments included a control diet based on corn-soybean without additives (T1), and the diet treatment with bacitracin (BMD 100 ppm, T2), colistin (10 ppm, T3), synbiotic (PoultryStar me, 0.5 kg/t, T4), a combination of synbiotic (0.5 kg/t) and bacitracin (60 ppm, T5), synbiotic (0.5 kg/t) and colistin (5 ppm, T6), synbiotic (0.5 kg/t), bacitracin (60 ppm), and colistin (5 ppm, T7). During the critical period of rearing from hatch to day 10, the synbiotic supplementation resulted in a significantly higher body weight gain than its combination with bacitracin. No other dietary treatment showed a remarkable improvement in the body weight gain, feed intake, or feed conversion ratio, compared to the only synbiotic application (T4) during the entire trial period. The tendency towards an improved feed conversion ratio was observed during the use of symbiotic (T4, 1.87), compared to the control group (T1, 1.93) during the entire trial period. Compared with the control group (T1, 2.78%), broiler mortality was also lower in the synbiotic group (T4, 1.11%). In the second experiment, a total of 1500 one-day-old male Ross 308 broiler chickens were randomly assigned to 4 diet treatments; with 15 replicates per diet treatment, and 25 birds per replicate over a 42-day period. The dietary treatments included a control group diet based on corn-soybean without additives (T1), and the treatment diets with bacitracin (BMD 1000 ppm, T2), synbiotic (PoultryStar me, 0.5 kg/t, T3), and a combination of synbiotic (0.5 kg/t) plus bacitracin (BMD 1000 ppm T4). Birds fed antibiotic or synbiotic alone or in a combination had numerically a higher body weight and an average daily gain than the control group. There was a tendency of improvement in the feed conversion ratio during the age of 1-24 days, and throughout the experimental period. The evaluated synbiotic could serve as an effective alternative to AGPs, such as bacitracin and colistin in broiler chicken diets, especially during the first crucial period. The synbiotic can serve this purpose without combining it with AGPs, such as colistin or bacitracin.

Keywords: Antibiotic growth promoter, Broilers, Performance, Synbiotic

INTRODUCTION

In view of the apprehensions of antibiotic resistance, probiotics have gained considerable interest in the poultry industry as alternatives to antibiotics (Gustafson and Bowen, 1997). Presently, this class of feed additives is largely used as an alternative to Antibiotic Growth Promoters (AGP) in poultry feeding. The main impetus that has catalyzed the use of these probiotic feed additives is the worldwide ban on the use of AGPs in the diets of food animals. The alternatives to AGPs should ideally possess the same beneficial effects as AGPs do possess when they are supplemented in the diet of food animals.

Despite the incredulous mechanism of action of the feed antibiotics (Huyghebaert et al., 2011), it is generally believed that the AGPs depict some antibacterial activities, which reduces the incidence and severity of subclinical infections, and decreases the microbial consumption of nutrients, thus improving the absorption of nutrients (Snyder and Wostmann, 1987; Brennan et al., 2003). The subsequent effect of all these activities leads to a better performance of the animal. The foundation of this explanation lies in the fact that AGPs do not exert growth-promoting effects in germ-free animals. The prevailing practice of the industry to feed livestock with sub-therapeutic doses of antibiotics is unlikely to have a growth inhibitory effect on the resident bacteria (Niewold, 2007). However, when antibiotics were added to the broiler diets at the levels below minimum inhibitory concentration, a clear shift in the intestinal microbiota was observed which at least partly explains the effects of AGPs (Pedroso et al., 2006; Wise and Siragusa, 2007).

Shifts in intestinal microbiota likewise affected the intestinal wall morphology and induced immune reactions which may promote the host animals' growth by affecting their energy expenses (Teirlynck et al., 2009). Thus, AGP-alternatives such as probiotics as the hypothetical AGP mode of action should also have modulatory effects on intestinal microbiota and immune system. Probiotics are live microorganisms that should be viable when they are administered in the livestock diets; in order to exert their beneficial effects on an improved intestinal function, intestinal microbiota balance, host immune responses, and the overall host health (FAO and WHO joint working group, 2002).

Dietary probiotics contribute to establish and maintain a beneficial intestinal microbiota, which may enhance the colonization resistance to pathogens, and strengthen the immune responses, leading to an improved growth performance (Dhama et al., 2011; Yang et al., 2012; Mountzouris, 2014; Mountzouris et al., 2015). The path of considering the gastrointestinal tract of food animals as the real complexity of anatomical system playing digestive, absorptive, metabolic, immunological, and endocrinological roles has progressed a lot in the last three decades (Oviedo-Rondon, 2019), the reason why the asseveration gut health became collectively important for the researchers and the livestock industry (Kogut et al. 2017).

The supplementation of probiotics and prebiotics has shown promising results in controlling bacterial infections in poultry by positively influencing the gut microbiota (Mead, 2000). Probiotics competitively excluded pathogenic microbes (Nava et al., 2005) and can be effective by stimulating the immune responses (Koenen et al., 2004), producing antibacterial substances, and stimulating digestive enzymes secretion (Saarela et al., 2000). Synergistic effects could be achieved through so-called synbiotics, a combination of probiotics and prebiotics (Roberfroid, 1998). The combined supplementation of poultry diets with probiotics and prebiotics (synbiotic) has been reported to be more effective than a single supplementation and in some cases even congruous with antibiotic treatments as reported in several studies and reviews (Gaggia et al., 2010; Gadde et al., 2017; Tayeri et al., 2018). Improvements in feed efficiency in broiler chickens as a result of synbiotic supplementation have been attributed to their potential modulatory effect on gastro-intestinal microbial colonization (Brugaletta et al., 2020). Prebiotics are indigestible carbohydrates supplemented frequently in combination with probiotics, which could stimulate the growth of useful bacteria in the intestines of the host (Lee et al., 2016). Prebiotic supplementation was shown to mimic the attachment sites of the pathogens, decreasing the adherence of pathogenic bacteria to the intestinal wall, and increasing specific beneficial bacteria (Ija and Tivey, 1998). Therefore, it draws a great interest to evaluate the effects of a synbiotic on the broiler chicken's performance.

The synbiotic product (PoultryStar me, Biomin Holding GmbH, Austria) evaluated in previous studies contained probiotic bacterial strains of *Enterococcus*, *Bifidobacterium*, *Pedicoccus*, and *Lactobacillus* species and a prebiotic fructooligosaccharide (Babazadeh et al., 2011).

Given the growth-promoting and immune-modulatory roles of AGPs (Niewold, 2007; Kogut and Swaggerty, 2012; Mountzouris, 2014), the performance response of the broilers to synbiotic products, when experimentally supplemented with AGPs in different combinations had been largely unknown. It was not clear whether there were additive effects due to the combination of AGPs and synbiotics.

The aim of these two experimental trials was therefore to evaluate the effect of dietary inclusion of a specific multi-species poultry synbiotic product alone or in different combinations with Bacitracin and/or Colistin, which are used as AGPs on the performance parameters in broiler chickens.

MATERIALS AND METHODS

Ethical approval

All procedures were performed in compliance with relevant laws and institutional guidelines. All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

First experiment

Animals and bird husbandry

A 42-day broiler feeding trial was conducted at the Poultry Research and Development Center of Kasetsart University in Kamphangsaen, Nakhon Pathom, Thailand with a total of 1261 day-old male Ross 308 broiler chickens (with an average body weight of 45 grams at 6-hours post-hatching), according to the prevailing institutional ethical norms. The chickens were weighed individually and assigned to seven treatment groups, each comprising of 6 replicates (n = 30 chickens on the first day). All chickens were raised in floor pens with rice husk as the litter material. Each compartment was equipped with manual feeders and bell-shaped drinkers without nipples. Feed (starter mash from day 1 to 10, grower mash from day 11 to 24, finisher mash 1 from day 25 to 35, and finisher mash 2 from day 36 to 42), and water were offered *ad libitum.* The lighting program was 23-hours light, and 1-hour dark period during the study. The chickens were housed in the evaporative cooling system during the experimental period. The chickens were vaccinated against Newcastle Disease (ND live B1) and Infectious Bronchitis on day 7, Infectious Bursal disease on day 14, and against Newcastle disease (La Sota strain) and Infectious Bronchitis on day 21 again. The temperature was maintained around 32 to 34°C for the first week, and then reduced weekly from 34°C to 25°C. The clinical observations regarding the animal health status, as well as the temperature, humidity, ventilation, and lighting of the trial house, were recorded daily during the experimental period.

Experimental diets and treatments

The trial chickens were randomly assigned to 7 dietary treatments. Each treatment consisted of 6 replications with 30 chickens per replication using a completely randomized design to minimize the effects of group compartments.

All experimental diets were based on the corn-soybean meal. The dietary treatments are presented in tables 1 and 2.

The ingredients and the chemical composition of the experimental diets are presented in table 3, and the nutrient composition of the experimental diets (proximate analysis) is presented in table 4. The synbiotic product used in the present study was obtained from Biomin Holding GmbH, Getzersdorf, Austria, and was included in the diet according to the manufacturer's recommendation. The multi-species product symbiotic (PoultryStar® me) contained probiotic bacterial strains of *Enterococcus*, *Pediococcus*, *Bifidobacterium*, and *Lactobacillus* species as well as a prebiotic fructooligosaccharide.

All the diets were analyzed (AOAC, 2016) for Dry Matter (DM, method 934.01), crude protein (method 988.05), Crude Fiber (method 962.09, CF, Foss Fiber Cap 2021 Fiber Analysis System, Foss Analytical, Hilleroed, Denmark), and crude fat (petroleum ether extraction; method 920.39). Feed samples of each experimental diet prepared for the trial were collected per phase and group immediately after blending and mixing.

Performance parameters measurement

Chicken live weight was recorded individually for each pen on the days 0, 10, twenty-four, and thirty-five, and per each group on the day forty-two. Body Weight Gain (BWG) was calculated per each group. Furthermore, Feed Intake (FI) was measured for the respective periods in combination with body weight measurements. Hence, the average of FI was determined for the respective periods per each group. Feed Conversion Ratio (FCR) for the respective periods was calculated for each group as the mortality-adjusted ratio between FI and BWG.

Second experiment

Animals and birds' husbandry

One thousand five hundred, one day old, male Ross 308 broiler chicks were divided into 4 dietary treatment groups. Each treatment comprised of fifteen replications with twenty-five chickens per replication, and the housing conditions were identical as in the first experimental trial.

Experimental diets and treatments

All diets were corn-soybean meal, formulated to meet the nutritional requirements recommended by Ross

308 nutrition specification guide as in the first experiment. The trial began when the birds were one-day-old, and it was finalized when they were forty-two days old. The chickens were divided into 4 dietary treatment groups. Each treatment consisted of fifteen replications with twenty-five birds per replication, followed by a fully randomized design to minimize the effects of group compartments. The dietary treatments are presented in table 2.

The ingredients and the chemical composition of the experimental diets are presented in table 5, and the nutritional composition of the experimental diets (proximate analysis) is presented in table 6. The synbiotic product used in the present study was similar to the first trial, obtained from Biomin Holding GmbH, Getzersdorf, Austria, and was included in the diet as recommended by the manufacturer. The multi-species product symbiotic (PoultryStar® me) contained probiotic bacterial strains of *Enterococcus*, *Pediococcus*, *Bifidobacterium*, and *Lactobacillus* species, and a prebiotic fructooligo-saccharide. All other details of the conditions and practices related to the preparations, mixing, and application procedures of the experimental diets were similar to those of the first trial.

Measurement of performance parameters

The procedures for measuring the performance parameters were the same as described for the first experiment.

Statistical analysis

The pens were the experimental units, and all data were pooled per pen, unless specified different and expressed as the mean, and pooled the Standard Error of Means (SEM). The data were subjected to a one-way analysis of variance (Statistical Package for Social Sciences, SPSS version 10.1) with the diets as the factor, and it was found to be significant. The means were separated by Duncan's new multiple range test at $p < 0.05$.

Table 1. Description of the Dietary treatments applied to Ross 308 broiler chickens

Treatment groups	Description
T1= Negative control (NC)	No additives in feed
T2 = Positive control (PC) 1 (AGP1)	Bacitracin (100 ppm* active ingredient).
T3 = Positive control (PC) 2 (AGP2)	Colistin (10 ppm active ingredient)
T4 = Synbiotic	Synbiotic (PoultryStar me 0.5 kg/ton of feed)
T5 = Synbiotic + PC 1 (PS**+AGP1***)	Synbiotic 0.5 kg/ton feed + Bacitracin (60 ppm active ingredient)
T6 = Synbiotic + PC 2 (PS+AGP2****)	Synbiotic 0.5 kg/ton feed + Colistin (5 ppm active ingredient)
T7 = Synbiotic + PC 1 + PC2 (PS+AGPs)	Synbiotic 0.5 kg/ton feed + Bacitracin (60 ppm active ingredient) + Colistin (5 ppm active ingredient)

*ppm: parts per million, **PoultryStar me, *** Antibiotic Growth Promoter 1 (Bacitracin), **** Antibiotic Growth Promoter 2 (Colistin)

Table 2. Description of the Dietary treatments applied to Ross 308 broiler chickens

Treatment groups	Description
T1= Negative control (NC)	No additives in feed
T2 = Positive control (PC) 1 (AGP)	Bacitracin (BMD*** 10% 1000 ppm*).
T3 = Synbiotic	PoultryStar 0.5 kg/t**** of feed
T4 = Synbiotic with AGP**	Poultry Star 0.5 kg / t + Bacitracin (BMD 10% 1000 ppm)

*ppm: parts per million, **Antibiotic Growth Promoter Bacitracin, *** Bacitracin methylene di-salicylate, **** kilogram per ton

Table 3. Ingredient composition and calculated analysis of experimental diets fed to the Ross 308 broiler chickens during the 42-day trial in the facility of Kasetsart University.

Ingredients	Unit	Starter (Day 1-10)	Grower (Day 11-24)	Finisher 1 (Day 25-35)	Finisher 2 (Day 36-42)
Corn	%	53.40	57.17	61.69	61.69
Soybean meal (46 % CP)	%	30.78	25.94	19.77	19.77
Full fat soybean (35.5 % CP)	%	12.00	13.50	15.00	15.00
Rice bran oil	%	0.50	0.50	1.24	1.24
MDCP (16.9 % Ca, 21.6 % P)	%	0.52	0.33	0.09	0.09
Limestone (38.7 % Ca)	%	0.96	0.87	0.72	0.72
Salt	%	0.41	0.41	0.39	0.39
Sodium bicarbonate (27 % Na)	%	0.05	0.05	-	-
Choline chloride (60 %)	%	0.04	0.03	0.04	0.04
Premix	%	0.60	0.60	0.60	0.60
L-Lysine	%	0.28	0.22	0.19	0.19
DL-Methionine	%	0.26	0.22	0.20	0.20
L-Threonine	%	0.13	0.09	0.06	0.06
Salinomycin (66 ppm)	%	0.05	0.05	0.05	-
Lutanox	%	0.02	0.02	0.02	0.02
Phytase	%	0.01	0.01	0.01	0.01
Total	%	100.00	100.00	100.00	100.00
Calculated analysis					
ME for poultry	kcal/kg	3053	3100	3200	3200
Protein	%	23.00	21.50	19.50	19.50
Fat	%	5.02	5.41	6.54	6.54
Fiber	%	4.00	3.90	3.72	3.72
Digestible Lysine (Poultry)	%	1.28	1.15	1.02	1.02
Digestible Methionine (Poultry)	%	0.51	0.47	0.43	0.43
Digestible Threonine (Poultry)	%	0.88	0.79	0.70	0.70
Lysine	%	1.44	1.30	1.17	1.17
Methionine + Cysteine	%	0.92	0.87	0.80	0.80
Methionine	%	0.61	0.55	0.50	0.50
Threonine	%	0.97	0.88	0.78	0.78
Calcium	%	0.80	0.72	0.61	0.61
Total phosphorus	%	0.60	0.55	0.48	0.48
Avail. Phosphorus (poultry)	%	0.33	0.29	0.24	0.24
Choline	%	1700	1600	1500	1500
Sodium	%	0.19	0.19	0.17	0.17
Salt	%	0.45	0.45	0.42	0.42

ME = Metabolizable Energy, MDCP = Mono-Dicalcium Phosphate.

Table 4. Nutrient composition of experimental diets (proximate analysis) fed to the Ross 308 broiler chickens during the 42-day trial in the facility of Kasetsart University.

Item	Period			
	Starter	Grower	Finisher1	Finisher2
Protein (%)	22.31	20.54	18.25	18.68
Fiber (%)	4.39	4.66	4.08	3.87
Fat (%)	6.01	6.26	5.49	6.08
Ash (%)	4.6	4.69	3.87	3.84
Calcium (%)	0.79	0.8	0.63	0.57
Phosphorus (%)	0.41	0.43	0.34	0.33
GE (kcal/kg)	4.657.52	4.684.73	4.530.39	4.603.69

GE = Gross Energy

RESULTS

First experiment's results

All chickens were healthy during the experimental period, and there was no mortality during the most critical period from the hatch to day 10. The outcome depicted that the synbiotic supplementation in the broiler diet resulted in a significantly higher Body Weight Gain (BWG) than its combination with bacitracin ($p<0.05$) during the hatch to day 10 (Table 7). Additionally, the treatment groups T3 (colistin alone) and T6 (colistin with synbiotic) resulted in a significantly better ($p<0.05$) BWG during this period compared to the control group (Table 7). None of the other treatments improved BWG, FI, or FCR significantly compared to the only synbiotic application (T4) during the entire experimental period from the hatch to day 42 (Table 8). An improved FCR (p = 0.0756) of 1.86 was observed in the bacitracin group (T2), 1.87 in the symbiotic group (T4), and 1.83 in the synbiotic-AGPs combination group (T7), respectively compared to the control group (T1, 1.93), and other treatment groups during the entire trial period (Table 8). No mortality was observed in the colistin-synbiotic combination group (T6) during the entire trial period. However, remarkably low mortality of 1.11% occurred in the bacitracin group (T2), the synbiotic group (T4), and the synbiotic-AGPs combination group (T7), respectively during the entire trial period compared to the control group (T1, 2.78%) and other treatment groups (Table 8).

Second experiment's results

The birds were healthy throughout the entire experimental trial. The crude protein contents in the mixed feeds corresponded to the calculated values. The amount of crude fat, crude fiber, Calcium (Ca), and phosphorous (P) in the experimental diets also was confirmed well by the calculated values (Table 5). Although no significant differences between the dietary treatments regarding zootechnical parameters were observed, the birds fed only with AGP or synbiotic and AGP in combination with synbiotic had a numerically higher body weight and average daily BWG than the non-supplemented control groups (p = 0.2500). This led to a tendency to improve FCR between the age of 1 to twenty-four days old, and throughout the experimental period of 1 to forty-two days (Tables 9 and 10).

Table 5. Ingredient composition and calculated analysis (%) of the second experimental diets fed to the Ross 308 broiler chickens during the 42-day trial in the facility of Kasetsart University.

Ingredient	Unit	Starter	Grower	Finisher
Corn	%	54.75	59.52	64.30
Soybean oil	%	1.92	1.72	1.50
Soybean Meal 48 %	%	30.65	24.48	18.25
Full fat Soybean	%	8.00	10.00	12.00
Calcium carbonate	%	1.45	1.33	1.22
MCP-22	%	1.79	1.60	1.44
Salt	%	0.36	0.36	0.36
DL-Methionine	%	0.34	0.30	0.26
L-Lysine	%	0.25	0.23	0.23
Threonine	%	0.09	0.07	0.04
Choline Chloride 60%	%	0.06	0.06	0.05
Antioxidant	%	0.01	0.01	0.01
Toxin Binder	%	0.15	0.15	0.15
Premix (vitamin + mineral)	%	0.18	0.18	0.18
Total	%	100.00	100.00	100.00
Calculated analysis				
ME for Poultry	kcal/kg	3100.00	3150.00	3200.00
Protein	%	23.00	21.00	19.00
Moisture	%	10.92	10.97	11.03
Fat	%	5.93	6.24	6.53
Fiber	%	3.15	3.20	3.26
Ash	%	5.80	5.31	4.86
Ca	%	0.96	0.87	0.79
Total P	%	0.77	0.71	0.65
P avail	%	0.48	0.44	0.40
Salt	%	0.36	0.35	0.35
Lysine	%	1.44	1.29	1.16
Methionine	%	0.67	0.61	0.55
Methionine + Cysteine	%	1.08	0.99	0.91
Threonine	%	0.97	0.88	0.78
Tryptophan	%	0.28	0.25	0.22
Arginine	%	1.54	1.39	1.24
Choline Chloride	mg/kg	1700.00	1600.00	1500.00

ME = Metabolizable Energy, MCP = Monocalcium Phosphate 22% feed grade

Table 6. Nutrient composition of the second experimental diets (proximate analysis) fed to the Ross 308 broiler chickens during the 42-day trial in the facility of Kasetsart University.

Nutrient (%) / Starter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Moisture	11.60	11.29	11.48	11.34
Protein	21.93	21.48	21.93	22.31
Fat	5.98	5.70	5.48	5.52
Fiber	2.38	2.44	2.40	2.39
Ash	5.81	5.77	5.72	5.69
Calcium	1.01	0.97	1.00	1.00
Phosphorus	0.80	0.74	0.78	0.77
GE (kcal/kg)	4092.82	4106.91	4174.68	4187.77
Grower				
Moisture	11.44	11.29	10.76	11.18
Protein	19.77	19.78	20.10	19.72
Fat	5.96	6.08	6.13	6.01
Fiber	2.03	2.06	2.15	1.95
Ash	6.23	6.32	6.31	6.16
Calcium	0.89	0.91	0.88	0.92
Phosphorus	0.71	0.74	0.73	0.71
GE (kcal/kg)	4147.89	4180.21	4238.82	4230.72
Finisher				
Moisture	12.01	12.02	11.75	11.70
Protein	18.90	18.78	18.98	18.67
Fat	6.70	6.65	6.40	6.52
Fiber	2.06	2.22	2.02	2.12
Ash	4.82	4.83	4.89	4.94
Calcium	0.80	0.82	0.82	0.79
Phosphorus	0.63	0.63	0.64	0.61
GE (kcal/kg)	4373.33	4385.00	4253.81	4218.67

GE = Gross Energy, ME = Metabolizable Energy

DISCUSSION

Presently, probiotics are largely used as alternatives to antibiotic growth promoters (AGP) in the modern poultry nutrition due to concerns of antibiotic resistance, and the ban imposed on the usage of AGPs in the diets of food animals. Beneficial effects of single or multi-species probiotics on the zootechnical performance of broiler chickens were increasingly documented in the scientific literature (Applegate et al., 2010; Fuentes et al., 2013; Zhang and Kim, 2014; Gadde et al., 2017; Tayeri et al., 2018). The data strongly suggested an improvement in health throughout the experimental period and the complete absence of mortality during the most critical period of day 0 to 10 (Table 7). This is in concordance with studies by Pelicano et al. (2004), and Takahashi et al. (2005), in which the use of different growth promoters in the early phase of rearing led to no differences in the viability and mortality rates of the broiler chickens. The present results indicated that the synbiotic supplementation in the diets of broiler chickens resulted in a significantly higher BWG than the combination with bacitracin ($p < 0.05$) during the first days of the post-hatch brooding period, considered the most critical phase of rearing from hatch to day 10 (Table 7). Probiotics are known to contribute towards the establishment and maintenance of a beneficial intestinal microbiota, which could enhance the colonization resistance to pathogens, and immune response improvements resulting in improved growth performance (Mountzouris, 2014; Mountzouris et al., 2015; Kogut et al. 2017; Baldwin et al., 2018; Oviedo-Rondon, 2019; Brugaletta et al., 2020).

The body weight gain was significantly better ($p < 0.05$) in T3 (colistin alone) and T6 (colistin with synbiotic) treatment groups compared to the control groups during this critical period from hatch to day 10 of age (Table 7). Synergistic effects were observed by feeding synbiotics, which are a combination of probiotics and prebiotics (Roberfroid, 1998; Gaggia et al., 2010; Gadde et al., 2017; Tayeri et al., 2018). There was not any significant improvement in BWG, FI, or FCR in any other group compared to the only synbiotic application (T4) during the entire study period from the hatching day to the day forty-second (Table 8).

An improved FCR (p=0.0756) of 1.86 was observed in the bacitracin group (T2), 1.87 in the synbiotic group

(T4), and 1.83 in the synbiotic-AGPs combination group (T7), respectively compared to the control group (T1, 1.93), and other treatment groups in the study (Table 8). No mortality was recorded in the colistin-synbiotic combination group (T6) during the entire trial period. In the bacitracin group (T2), the synbiotic group (T4), and the synbiotic-AGPs combination group (T7), however, a very low bird mortality rate of 1.11% occurred compared to the control group (T1, 2.78%), and other treatment groups (Table 8). Chickens in the second experimental trial were also healthy during the entire study. Although no significant differences among the dietary treatments regarding zootechnical parameters were observed, birds fed with AGP or synbiotic alone, and their combination, had a numerically higher body weight and average daily BWG than that of the control groups (p=0.2500). This improvement in the BWG in these treatment groups tended to improve FCR in the chickens aged 1 to twenty-four days, and 1 to forty-two days old throughout the experimental period (Tables 9 and 10). No significant differences in body weight, FI, FCR, and mortality among the synbiotic, colistin, and bacitracin groups alone or in combination with each other revealed that AGP could be replaced by synbiotics without loss of zootechnical performance.

Table 7. Effect of the combination of synbiotics with antibiotic growth promoters on the production performance of broiler chickens from day of hatch to day 10.

Treatment groups[1]	BWG (g/bird)	FI (g/bird)	FCR	Mortality (%)
T1	192.656 [ab]	263.472	1.36	0.00
T2	193.094 [ab]	272.611	1.41	0.00
T3	199.133[a]	271.389	1.36	0.00
T4	198.461[a]	270.217	1.36	0.00
T5	186.050[b]	272.361	1.46	0.00
T6	199.678[a]	267.611	1.34	0.00
T7	193.189 [ab]	265.583	1.37	0.00
p -value	0.0337	0.6047	0.1492	0.00
SEM	1.2663	1.5107	0.0129	0.00

[a,b] Means with dissimilar letters in a column varied significantly ($p < 0.05$) [1] T1= No additives in feed, T2 = Bacitracin, T3 = Colistin, T4 = Synbiotic (PoultryStar® me), T5 = Synbiotic + Bacitracin, T6 = Synbiotic + Colistin, T7 = Synbiotic + Bacitracin + Colistin. BWG = Body Weight Gain, FI = Feed Intake, FCR = Feed Conversion Ratio, SEM = Standard Error of Means

Table 8. Effect of the combination of synbiotics with antibiotic growth promoters on the production performance of broiler chickens from the first day to day 42.

Treatment groups[1]	BWG (g/bird)	FI (g/bird)	FCR	Mortality (%)
T1	2872.50	5547.01	1.93	2.78
T2	2921.17	5435.59	1.86	1.11
T3	2871.02	5540.05	1.93	1.67
T4	2923.90	5479.02	1.87	1.11
T5	2883.07	5412.70	1.88	1.67
T6	2893.12	5444.65	1.88	0.00
T7	2972.42	5453.08	1.83	1.11
p-value	0.7736	0.6241	0.0756	0.4758
SEM	18.1249	22.4774	0.0096	2.2160

[1] T1= No additives in feed, T2 = Bacitracin, T3 = Colistin, T4 = Synbiotic (PoultryStar® me), T5 = Synbiotic + Bacitracin, T6 = Synbiotic + Colistin, T7 = Synbiotic + Bacitracin + Colistin. BWG = Body Weight Gain, FI = Feed Intake, FCR = Feed Conversion Ratio, SEM = Standard Error of Means.

Table 9. Effect of dietary treatments on growth performance of Ross 308 broiler chickens from day 1 to day 24, fed in the facility of Kasetsart University.

Treatment	Feed intake (g)	Body weight (g)	ADG (g/bird/day)	FCR	% Livability
NC	1720.573	1013.843	42.243	1.698	99.733
AGP	1717.280	1021.277	42.553	1.682	100.000
PS	1721.093	1021.189	42.550	1.687	99.733
PS + AGP	1721.107	1020.827	42.534	1.686	99.733
p-value	0.9818	0.8752	0.8752	0.6611	0.8013
SEM	3.7512	3.7136	0.1547	0.0047	0.1135

NC = negative control, no additives in feed, PS = PoultryStar® me, AGP = antibiotic growth promoters, Bacitracin, ADG = average daily weight gain, FCR = feed conversation ratio, SEM = Standard Error of the mean, g = gram

Table 10. Effect of dietary treatments on growth performance of Ross 308 broiler chickens from day 1 to day 42, fed in the facility of Kasetsart University.

Treatment	Feed intake (g)	Body weight (g)	ADG (g/bird/day)	FCR	Livability (%)
NC	4740.160	2536.299	61.403	1.841	99.200
AGPs	4722.093	2572.408	62.196	1.811	99.733
PS	4724.067	2548.600	61.697	1.824	99.467
PS + AGPs	4751.960	2565.667	62.103	1.824	99.733
p -value	0.8165	0.3723	0.3732	0.2500	0.6368
SEM	12.4302	20.4605	0.4876	0.0115	0.1672

NC = negative control, no additives in feed, PS = PoultryStar® me, AGP = antibiotic growth promoters, Bacitracin, ADG = average daily weight gain, FCR = feed conversation ratio, SEM = Standard Error of the mean, g = gram

CONCLUSION

Overall, the results of these two experiments under the controlled conditions proved that the evaluated synbiotic (PoultryStar® me) could serve as a replacement and an effective alternative to the Antibiotic Growth Promoters (AGPs), such as bacitracin and colistin in the broiler diets. With careful evaluation and the right preventive programs, the synbiotic can serve this purpose without being combined with AGP's. Hence, the replacement could be cost-effective and bring more value to broiler chicken producers.

DECLARATIONS

Authors' contribution

The experimental studies were conceived and designed by Basharat Syed and Yuwares Ruangapanit in consultation with Silvia Wein. Yuwares Ruangapanit supervised the experimental trials, collection of data, and its analysis. Silvia Wein reviewed the statistical analysis. The manuscript was written and drafted by Basharat Syed. All authors read and approved the final manuscript for submission and publication.

Competing interests

The authors declare that they have no competing interests.

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